USAN 09/760,379
Attachment #1

Additional example:

The following peptides (peptide 1 - 8) were purchased from Bachem (Germany): human angiotensin I and II, substance P-methylester, neurotensin (clip 1-11), neurotensin, ACTH (clip 1 - 17), ACTH (human clip 18 – 39), and somatostatin. Solutions containing **(A)** 50 femtomol/µl and **(B)** 10 femtomol/µl of each of the peptides in 0.1 % trifluoroacetic acid (TFA) were prepared following the quantity specifications provided by the manufacturer. In two separate reaction vials (0.6 ml Eppendorf tubes, Eppendorf, Hamburg, Germany), 150 µl of the solution (A) and (B) were purified as follows:

Firstly, to each vial 125 µg of magnetic particles (nanomag® - D, silica, diameter: 250 nm, 50% derivatized with C-18-groups) suspended in 10 µl 0.1 % TFA were added. The particles were collected on one site of the wall of the vials by moving the vials close to a strong permanent magnet (Fe/Nd/B-magnet, 1.4 T, from IBS-Magnet, Berlin, Germany). While in this position (see Figure 1), the supernatants were replaced by 50 µl of 60% acetonitrile/0.1% TFA solution. The particles were resuspended and collected again as described above. Hereafter, the particles were washed with 50 µl of 0.1% TFA solution as described above. The two supernatants were replaced by the peptide solutions (A) and (B). The particles were resuspended and, after 3 min incubation time, collected as described above. The supernatants were replaced by 50 µl of 0.1% TFA solution, in which the particles were washed as described above. After removal of the washing supernatant, the particles were resuspended (the bound peptides eluted) in 10 µl of 60% acetonitrile/0.1% TFA solution. 0.3 µl of each supernatant, of the peptide solutions before purification, of the binding supernatants (peptides not bound to the particles) and of the washing supernatants were analyzed by MALDI-TOF-MS using the dried droplet sample preparation technique with α-cyano-4-hydroxycinnamic acid as the matrix, as described in: Kussmann, M., E. Nordhoff, H. R. Nielsen, S. Haebel, M. R. Larsen, L. Jakobsen, J. Gobom, E. Mirgorodskaya, A. K. Kristensen, L. Palm and P. Roepstorff, MALDI-MS sample preparation techniques designed for various peptide and protein analytes, J. Mass Spectrom., 32 (1997) 593-601. The

prepared samples were analyzed on a Bruker Scout MTP Reflex III MALDI-TOF mass spectrometer in reflector mode using delayed ion extraction and the acquisition parameters for peptides provided by the manufacturer. Exclusively positively charges ions were analyzed and 150 single-shot spectra were accumulated for improved signal-to-noise-ratio.

For the peptide solutions (A) and (B), the recorded mass spectra are compared in Figure 2.

It is clearly visible how the magnetic concentration and purification improved the quality of the mass spectrometric analyses, especially for peptide solution (C) containing only 10 femtomol/μl of each of the eight peptides. If more than 90% of the hydroxyl groups present on the surface of the nanomag® - D (silica, diameter: 250 nm) particles used for purification, were derivatized with C-18 groups, the particles agglutinated in 0.1% TFA, rendering the magnetic purification described above, impossible.

nanomag®-D silica, 250 nm	
Type:	silica fortified magnetite dextran composite
Nominal size:	250 – 350 nm
Shape:	cluster typed
Density:	4.0 g / cm³
Magnetization:	43 emu / g particles (H = 1000 Oe)
Saturation magnetization:	> 67 emu / g particles (H > 10.000 Oe)

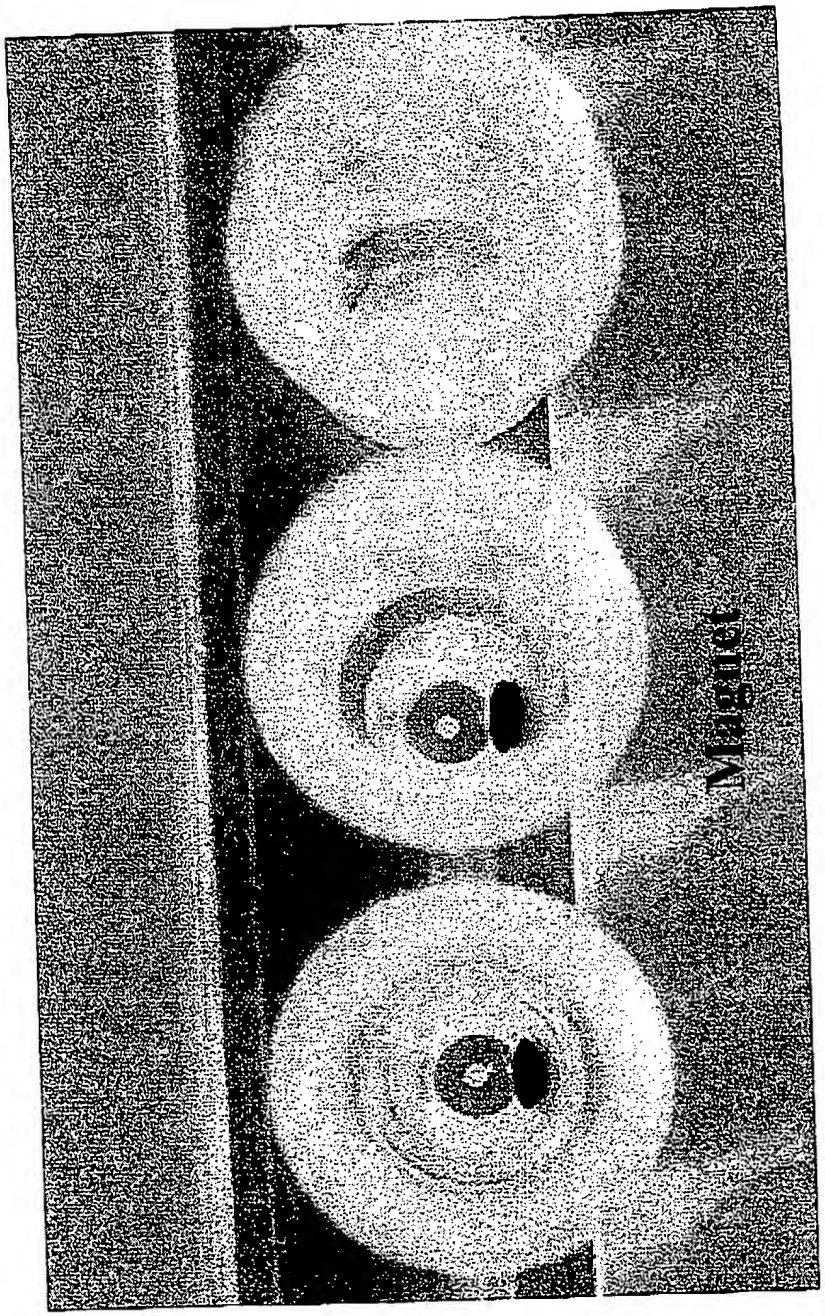

Figure 1: Magnetic particles (nanomag® - D, silica, diameter: 250 nm, 50% derivatized with C-18-groups) were suspended in 50 µl 0.1% TFA solutin, and subsequently collected by close contact to a strong permanent magnet.

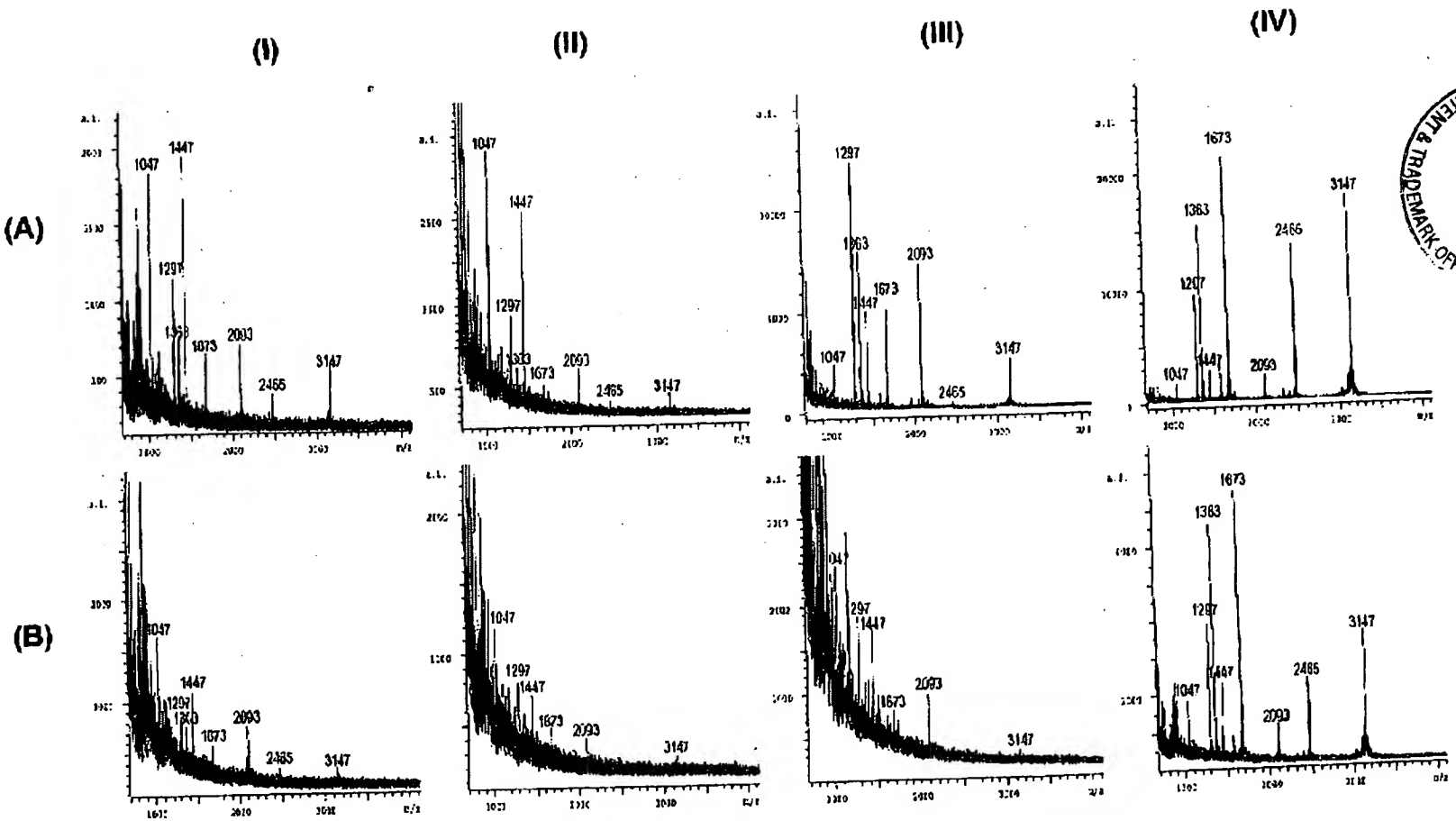

Figure 2: MALDI-TOF-MS mass spectra recorded from 0.3 µl of (I) peptide solution (A) and (B), (II) of the corresponding supernatants after incubation with magnetic particles (nanomag® - D, silica, diameter: 250 nm, 50% derivatized with C-18-groups), (III) of the corresponding washing supernatants, and (IV) of the purified peptide solutions.